Application No.: 10/583,280 Docket No.: 58086-232451

Amendment and Response to Restriction Requirement dated June 29, 2009

Response to Restriction Requirement of February 27, 2009

Amendments to the Specification:

Please replace paragraph [00063] with the following amended paragraph:

[00063] Typical DNA constructs were prepared as follows: pCSUACG (U6-shRNAαAR; CMV-GFP) was constructed by ligating the *BamHI/Eco*RI digests of pCSCG and the U6-shRNAαAR PCR product. The U6-shRNAαAR PCR was performed using a hU6-containing plasmid at a 60°C annealing temperature with suitable primers: pCSCA (CMV-AR) was created by subcloning the *Xba*I fragment of pSRα-AR into the *Nhe*I site of pCSCG. AR mutants were made by standard PCR-based site-directed mutagenesis using the QuikChange Kit (Stratagene). = NLS ΔNLS contains three point mutations (K618M, K632M, K633M) previously shown to disrupt nuclear import. ⁴⁹ = Pro ΔPro contains a deletion of amino acids 372-381, based on prior work. ³² ARR₂Pb-Luciferase was kindly provided by Robert Matusik (Vanderbilt). *PSA* RT-PCR was also performed using suitable primers.

Please replace paragraph [00064] with the following amended paragraph:

Details of typical *In vitro* and *In vivo* Growth *in vitro* and *in vivo* growth experiments are as follows: LNCaP (ATCC) and LAPC4 cells were maintained in Iscove's medium supplemented with 10% fetal bovine serum. LNCaP-AR and LNCaP-vector were derived by infection with the pSR_□ AR or pSR_□ pSRα-AR or pSRα retrovirus, respectively, and selection in 500 ng/ml of G418. LNCaP-AR, LNCaP-vector, LAPC4-AR, and LAPC4-vector in other experiments were derived by infection with the pCSCA or pCSC lentivirus, respectively, without selection (>90% infection). For *in vitro* experiments, LNCaP or LAPC4 cells stably infected with different constructs were androgen-starved by growth in charcoal-stripped serum for 3-5 d. 5x10⁴ cells were plated per well in media containing 10% charcoal-stripped serum supplemented with various concentration concentrations of R1881 or in media containing 10% full serum with various concentration concentrations of bicalutamide. Colonies were visualized with crystal violet staining 2 weeks later. *In vivo* tumorigenicity was measured by injection of 5 x 10⁵ LAPC4 or 1 x 10⁶ LNCaP cells in 100 μl of Matrigel (Collaborative Biomedical) subcutaneously into the flanks of intact or

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castrated male SCID mice. Tumor size was measured weekly in three dimensions using caliber as described. AR knockdown was performed by infection of HR LAPC4 with shRNA AR lentivirus. Tumors which grew in castrated mice were explanted, and analyzed by flow cytometry for the percentage of GFP-positive cells. All mouse experiments were performed in compliance with the guidelines of the Animal Research Committee (ARC) of the UCLA.